TECHNIQUES

Herpetological Review, 2013, 44(2), xxx–xxx.

The Use of Visible Implant Alpha Tags for Anuran Tadpoles

Amphibians are one of the most threatened groups of vertebrates in the world and are currently facing major declines due to habitat loss, emerging infectious diseases, and climate change, leading many species to extinction (Stuart et al. 2004). To face this amphibian extinction crisis, there is an urgent need to gather data on the demography and ecological characteristics of amphibian species (Wake 2007). In most of these studies, it is necessary to be able to identify individuals across time to study density, growth trajectories, or habitat use. Date specific cohort marks can be used for density estimates but when intending to quantify growth, movement or survival, marks have to allow identifying individuals. Such marks have to possess a set of characteristics. First, it should allow each individual to be identified accurately. Second, it should have a minimal impact on the individual and should not affect behavior, survivorship and catchability.

A variety of techniques are available for individual recognition in amphibians. For adult anurans, toe clipping was the method most commonly used since the 1950s (Martof 1953). It has been shown to be well adapted and to have little impact for some amphibian species (Grafe et al. 2011) and studies focusing on physiological indicators of distress show no increased stress resulting from toe-clipping (Perry et al. 2011). One of the drawbacks of toe-clipping is that it may have an effect on recapture and survival for some species (McCarthy and Parris 2004). More recently, Passive Integrated Transponder (PIT tag) have been proposed for amphibians (Brown 1997) and proved to be particularly well suited. Nonetheless, PIT tags are still limited in their use by their size and can only be inserted in adult amphibians of larger size (4 cm SVL). The use of color patterns via picture identification (Kenyon 2009) has also been proposed as an alternative technique for the identification of adult amphibians but requires a specific color pattern for each individual, as e.g. found in tropical amphibian species. Finally, Visual Implant Elastomers (VIE) may mainly be used for date specific cohort marking. To allow individual recognition, VIE marks have to be placed in distinct body parts or to be coupled with toe clipping (Hoffmann et al. 2008). Several marking procedures have been employed in studies of larval amphibians including tail-clipping (Turner 1960), staining of the entire tadpole (Travis 1981), coded wire tags (Martin 2011) and picture identification (Ribeiro and Rebelo 2011). Nonetheless, few of these techniques are suitable for monitoring the entire lifespan of anurans from the tadpole to the adult stage.

One alternative technique to mark amphibians both at the adult and larval stage is VI Alpha tagging (© Northwest Marine Technology Incorporation). VI Alpha tags are small rectangles of an inert elastomer inscribed with an alphanumeric code. Tags are available in four colors providing 2600 unique alphanumeric codes. VI Alpha tags were initially developed for fishes (Karvonen et al. 2004), but they have been successfully used with others organisms such as shrimp (Arce et al. 2003), or seahorses (Woods 2005). In the last five years, VI Alpha tags have been tested successfully for use on caecilians (Gower et al. 2006; Measey et al. 2001), urodeles (Osbourn et al. 2011), and anurans (Chelgren et al. 2006; Heard et al. 2008; Pittman et al. 2008). None of these studies were able to assess whether VI Alpha tags can allow following an individual through metamorphosis.

In this study, we evaluated the use of VI Alpha tags to mark tadpoles of the anuran species *Alytes obstetricans* and tested whether the mark remains after metamorphosis. More specifically, we aimed to assess whether these tags can be used for ecological studies using individual recognition in *A. obstetricans* by determining: 1) the rate of tag loss, 2) the readability of the tag across tadpole development until metamorphosis, and 3) the effect of such tags on survival rate.

ELODIE A. COURTOIS[1]
CÉLIA LELONG[1]
OLIVIER CALVEZ[1]
ADELINE LOYAU[1]
DIRK S. SCHMELLER[1]*

[1]*Station d'Ecologie Expérimentale du CNRS à Moulis, USR 2936, 09200 Moulis, France*
**Corresponding author; e-mail: ds@die-schmellers.de*

Materials and Methods

Study species.—*Alytes obstetricans*, the Common Midwife Toad, is a European species ranging from northern Portugal and Spain in the south to southern Belgium and Central Germany to the northeast. It is present throughout France, except in the high Alps. It ranges from the sea line up to altitudes of 2400 m in the Pyrenees. This species is known for its male parental care behavior. The male carries a string of eggs wound on its hind legs until they are ready to hatch. After three to six weeks, the male releases the hatching larvae into suitable water habitat. Tadpoles measure about 15mm just after hatching, but can reach up to 10 cm in total length before metamorphosis with a mean body size of 2.3 (Standard Deviation = 0.4 cm; Courtois et al., unpubl. data).

VI Alpha tags.—VI Alpha tags (© Northwest Marine Technology Incorporation) are 1.2 × 2.7 mm tags (Fig. 1) that are

subcutaneously inserted with a specific injector needle. Each tag is coded with black letters and numbers on a fluorescent colored background. In this study, we used tags with a yellow background. After tagging, the unique code remains visible to the naked eye under day light and readability can be enhanced with UV-light (delivered by © Northwest Marine Technology Incorporation; Fig. 1).

We collected 90 tadpoles at Gosner Stage 25 (Gosner 1960) from a large population in a trough in Balagué (Ariège, France, 42.963889°N, 1.025278°E) measuring 5.3 ± 0.7 cm in total length (including tail) and weighting 1.5 ± 0.5 g. We tagged 30 tadpoles on the ventral side and 30 dorsally, resulting in 60 tadpoles marked in total. In half of the cases (15 tadpoles in each group), we used a surgical glue to help healing and potentially prevent tag loss.

Tadpoles were first immobilized by immersion in ice-cold water (around 4°C) and were restrained by gently squeezing the body between the thumb and index finger. A 3 µm wide needle was used to make a small first incision in the epidermis and the tag was inserted under the skin using the supplied needle injector. Whilst this instrument is designed for direct injection of VI Alpha tags, prior creation of a skin incision expedited the injection process considerably (Heard et al. 2008). Tadpoles were handled with powder-free latex gloves during the tagging manipulation but no gloves were used in subsequent manipulation. We did not experience any tadpole mortality related to the usage of Latex gloves as found in other studies (Cashins et al. 2008), neither in the experiment described here nor in field research done on the same species (Schmeller et al., unpubl. data).

VIE tags.—We marked 30 tadpoles with VIE (© Northwest Marine Technology Incorporation) tags (orange) in the back in order to compare mark loss and survival rate between these two tag types. VIE are biologically compatible polymers which are composed of a colorant and a curing agent. They are mixed in equal quantity just before use, resulting in a liquid. The liquid is then sucked into a syringe and injected dorsally under the skin of the tadpole, where it solidifies to a permanent mark. The mark remains visible with the naked eye, but visibility can be further increased using UV-light.

Tadpole rearing.—The 90 tadpoles were kept together in an aquarium with constantly filtered and aerated water at 18°C, pH 6.9, and a photoperiod of 10 hours of daylight. The aquarium was cleaned and water was replaced once a week. The tadpoles were fed after water replacement with fish food tablets (Tetra TabiMin). They were kept for six weeks in order to monitor both tadpole survival, tag loss until metamorphosis and readability after metamorphosis. Once a week we recorded whether the VI Alpha tag or the VIE tag was still present or not for each individual. We defined tag retention as the percentage of tags in each group that were still present at the end of the experiment (i.e., 42 days after marking). For the VI Alpha tag, when the tag was present, we noted its degree of readability (index 0: not readable, index 2: readable only with UV-light, and index 3: readable under daylight), and the Gosner stage of the tadpole (Gosner 1960). The Gosner stage varied from 25 (no limb bud) to 46 (complete metamorphosis). When the tag was lost, the tadpole was released back into the wild. When the tag was retained, the tadpole was released after completion of metamorphosis, usually after five to six weeks.

Statistical analysis.—Binomial generalized linear models (GLM) were run to explain tag retention at the end of the experiment as a function of place of injection (ventral or dorsal) and use of glue, using the software package R (http://cran.r-project.org/). The variable "presence" was binary, as either the tag was still present (1) or lost (0). Similarly, the same type of model was used to determine the effect of the factors "place of insertion" (dorsal or ventral) and "surgical glue" (use or not) on the readability of the VI Alpha tags. Readability was then defined as 0 when not readable (index 0) and 1 when readable either with day light (index 3 defined previously) or UV-light (index 2 defined previously).

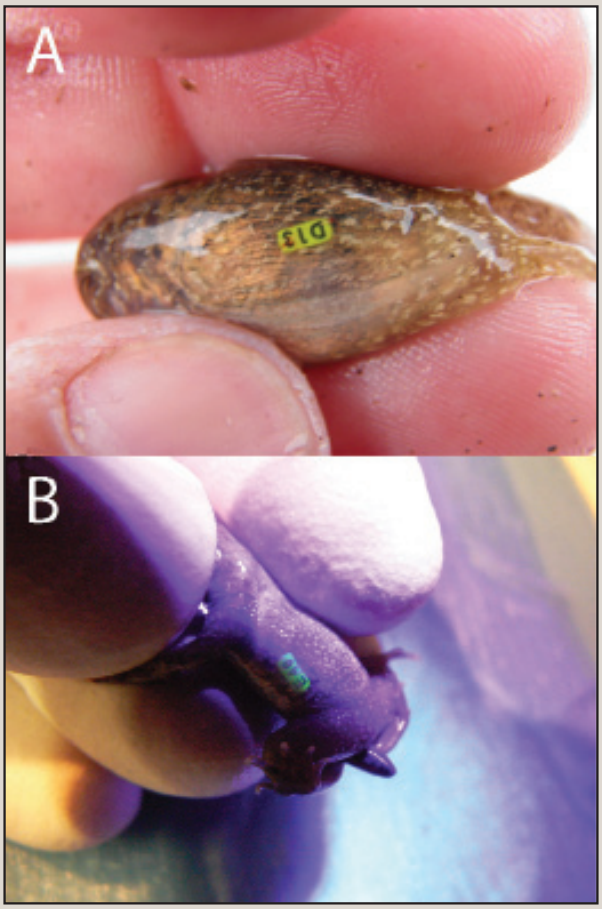

FIG. 1. VI Alpha tag inserted on the ventral side of *Alytes obstetricians* for a (A) tadpole and (B) recently metamorphosed frog when enhanced with UV-light.

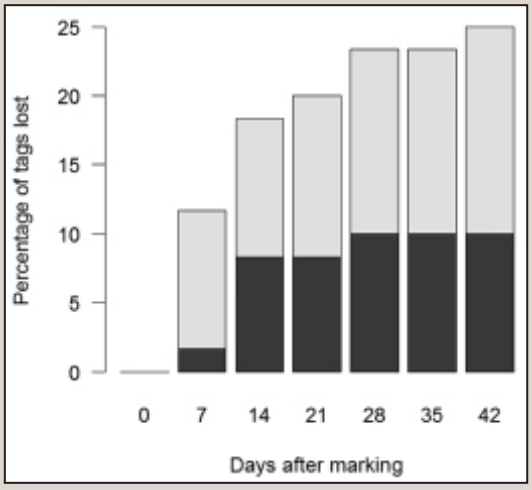

FIG. 2. Cumulative percentage of tags lost out of 60 as a function of the number of days after the injection when injected ventrally (in light grey) or dorsally (in dark grey).

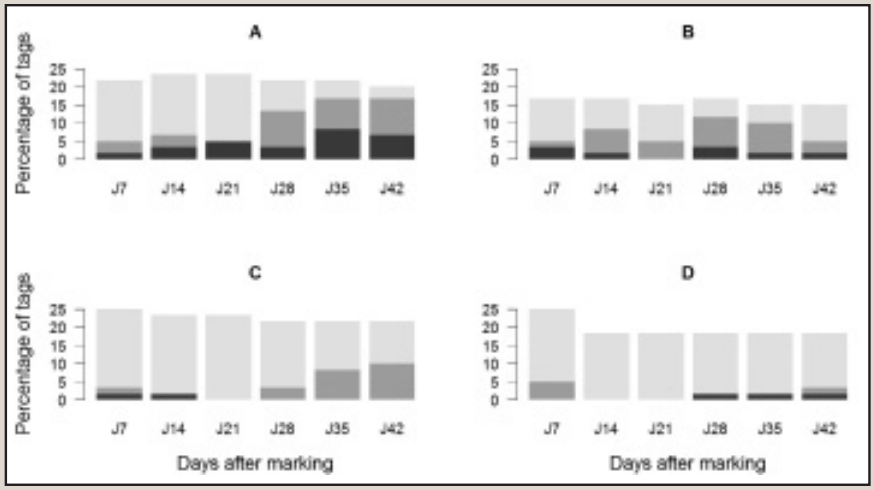

FIG. 3. Percentage of tags out of 60 that were not readable (black), readable only with UV light (dark grey) and readable under daylight (light grey) for (A) tadpoles marked dorsally without glue, (B) tadpoles marked dorsally with glue, (C) tadpoles marked ventrally without glue and (D) tadpoles marked ventrally with glue.

TABLE 1. Readability (% of readable tags—i.e., with readability index 2 or 3—at the end of the experiment) and tag retention (% of remaining tags at the end of the experiment) 42 days after marking).

	Readability (%)	Final tag retention (%)
Group A (dorsal, without glue)	67	80
Group B (dorsal, with glue)	89	60
Group C (ventral, without glue)	100	87
Group D (ventral, with glue)	91	73

RESULTS

In total, 15 tadpoles (25%) of the 60 individuals tagged with VI Alpha tags lost their tag before metamorphosis of which 6 were marked ventrally and 9 dorsally. Tag retention tended therefore to be higher on the ventral than on the dorsal side (Table 1) but this difference was not significant (GLM, Deviance = 0.15, P = 0.38). Of the 15 tags that were lost, 10 were sealed with surgical glue and 5 were not, but the difference between groups was not significant (GLM, Deviance = 0.41, P = 0.14). Tag loss was most important in the dorsal group during the first 7 days (6 out of 9 tags lost), while tag loss in the ventral group was later with a maximum during the second week (5 out of 6 tags lost; Fig. 2). The retention rate of VIE marked individuals was 100%.

Overall, 100% of VI Alpha tags were readable immediately after injection and an average of 87% were readable at the end of the experiment (Table 1). However, in 15 out of 45 cases UV light was needed to identify the VI Alpha tag code (readability index 2, Fig. 3). Our results show a higher readability on the ventral side than on the dorsal one (Table 2, GLM, Deviance = 0.43, P = 0.046). The better readability of the ventral tags compared to the dorsal tags was stable over tadpole development (Fig. 2). The use of surgical glue did not affect the readability of the tags (GLM, Deviance = 0.03, P = 0.58).

No tadpoles died during the experiment; the survival rate under controlled condition was therefore 100% for both the VIE tag group and the VI Alpha tag group.

DISCUSSION

Here, we tested the usage of VI Alpha tags for the marking of tadpoles of the species *A. obstetricans* under laboratory conditions. We found that tag retention was globally low (75%), rendering this technique unsuitable for Capture-Mark-Recapture studies on this species by introducing tag-loss bias. A previous study on the use of VI Alpha tags on amphibians demonstrated a level of 92.31% of tag retention for *Litoria raniformis* (Heard et al. 2008), which is comparable to the tag retention in the group in which we inserted VI Alpha tags ventrally and used no surgical glue to close the insertion (87%). Indeed, contrary to our expectations, the use of surgical glue did not increase the retention rate and seems to be more painful for tadpoles even under cold anesthetic (Calvez and Lelong personal observation) and we therefore advise against its use. To counterbalance tag loss, Heard et al. (2008) proposed to couple the use of small VIE dot marks with VI Alpha tags in order to be able to identify an individual as marked even if the VI Alpha tag was lost, a suggestion also valid for *A. obstetricans* tadpoles as the VIE-retention was 100% in our study.

The retention rate of the VI Alpha tags tended to be higher on the ventral than on the dorsal side, and additionally, the readability of the tags was significantly higher on the ventral side. Hence, ventral insertion of the VI Alpha tags should be preferred in future studies, especially as VI Alpha tags were readable after metamorphosis. Future studies should assess whether VI Alpha tags are readable in adult frogs that were marked as tadpoles. If so VI Alpha tags may be useful in studies on breeding site fidelity, home range size, dispersal distances, infection load and habitat selection of amphibians (Pittman et al. 2008). Nonetheless, it should be noted that *A. obstetricans* tadpoles are especially large and tadpole marking in other species may not always be possible with this technique.

One advantage of VI Alpha tag compared to VIE is that only one injection is needed, as compared to multiple injections necessary for individual recognition using VIE-markers. Hence, the marking effort is strongly reduced per marking and recapturing sessions, allowing for an increased number of marked individuals and limiting the risk of mistaken identity. Readability of tags remains high (87%) even at the end of the experiment. Decrease of readability during the development of the tadpole and after the metamorphosis can be explained by the fact that tadpole skin becomes less transparent in tadpole of high Gosner stage and even less in post-metamorphic tadpoles. VI Alpha tags are also a good alternative to more expensive marking techniques such as PIT tags. It opens up the possibility to work with smaller-sized amphibian species and larval stages as the minimum size for VI Alpha tags is 2 cm for SVL compared to 4 cm for PIT tags. Moreover, Alpha tags are flatter than PIT tags. Further, survival rate was 100% and we therefore agree with the assessment of Heard et al. (2008) that VI Alpha tags are a promising alternative to PIT tags for marking larval amphibians.

Future studies should be conducted to determine whether the results observed in this study are the same under field conditions. Moreover, marking could change the subsequent behavior

of tadpoles and make them more susceptible to disease or predation in the field (i.e. reduce their survivorship under field conditions). A field study may therefore be able to conclude on the use of VI Alpha tag for *A. obstetricans* tadpoles marking.

Acknowledgments.—This work was supported by grants from the BiodivERsA project RACE (Risk Assessment of Chytridiomycosis to European amphibian biodiversity; www.bd-maps.eu). We also thank Pieter Matthieu for help with the experiment. Authors have complied with all applicable Animal Care guidelines, and all required French permits (capture permit and authorization for experimentation with animals) have been obtained. We thank one anonymous reviewer for useful comments on earlier version of this manuscript.

Literature Cited

Arce, S. M., B. J. Argue, D. A. Thompson, and S. M. Moss. 2003. Evaluation of a fluorescent, alphanumeric tagging system for penaeid shrimp and its application in selective breeding programs. Aquaculture 228:267–278.

Brown, L. J. 1997. An evaluation of some marking and trapping techniques currently used in the study of anuran population dynamics. J. Herpetol. 31:410–419.

Cashins, S., R. Alford, and L. Skerratt. 2008. Lethal effect of latex, nitrile, and vinyl gloves on tadpoles. Herpetol. Rev. 39:298–301.

Chelgren, N. D., D. K. Rosenberg, S. S. Heppell, and A.I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecol. Appl. 16:250–261.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Gower, D. J., O. V. Oommen, and M. Wilkinson. 2006. Marking amphibians with alpha numeric fluorescent tags—caecilians lead the way. Herpetol. Rev. 37:302.

Grafe, T. U., M. M. Stewart, K. P. Lampert, and M. O. Rödel. 2011. Putting toe clipping into perspective: a viable method for marking anurans. J. Herpetol. 45:28–35.

Heard, G. W., M. P. Scroggie, and B. Malone. 2008. Visible implant alphanumeric tags as an alternative to toe-clipping for marking amphibians—a case study. Wildlife Res. 35:747–759.

Hoffmann, K., M. E. McGarrity, and S. A. Johnson. 2008. Technology meets tradition: a combined VIE-C technique for individually marking anurans. Appl. Herpetol. 5:265–280.

Karvonen, A., P. Hudson, O. Seppälä, and E. T. Valtonen. 2004. Transmission dynamics of a trematode parasite: exposure, acquired resistance and parasite aggregation. Parasitol. Res. 92:183–188.

Kenyon, N., A. D. Phillott, and R. A. Alford. 2009. Evaluation of the photographic identification method (PIM) as a tool to identify adult *Litoria genimaculata* (Anura: Hylidae). Herpetol. Cons. Biol. 4:403–410.

Martin, R. A. 2011. Evaluating a novel technique for individual identification of anuran tadpoles using coded wire tags. Herpetol. Cons. Biol. 6(1):155–160.

Martof, B. 1953. Home range and movements of the green frog, *Rana clamitans*. Ecology 34:529–543.

McCarthy, M. A., and K. M. Parris. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. J. Appl. Ecol. 41:780–786.

Measey, G. J., D. J. Gower, O. V. Oommen, and M. Wilkinson. 2001. Permanent marking of a fossorial caecilian, *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). J. South Asian Nat. Hist. 5:109–115.

Osbourn, M. S., D. J. Hocking, C. A. Conner, and W. E. Peterman. 2011. Use of fluorescent visible implant alphanumeric tags to individually mark juvenile ambystomatid salamanders. Herpetol. Rev. 42:43–47.

Perry, G., M. C. Wallace, D. Perry, H. Curzer, and P. Muhlberger. 2011. Toe clipping of amphibians and reptiles: science, ethics, and the law. J. Herpetol. 45:547–555.

Pittman, S. E., A. L. Jendrek, S. J. Price, and M. E. Dorcas. 2008. Habitat selection and site fidelity of cope's gray treefrog (*Hyla chrysoscelis*) at the aquatic-terrestrial ecotone. J. Herpetol. 42:378–385.

Ribeiro, J., and R. Rebelo. 2011. Survival of *Alytes cisternasii* tadpoles in stream pools: a capture-recapture study using photo-identification. Amphibia-Reptilia 32:365–374.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Travis, J. 1981. The effect of staining on the growth of *Hyla gratiosa* tadpoles. Copeia 1981:193–196.

Turner, F. B. 1960. Population structure and dynamics of the western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone park, Wyoming. Ecol. Monogr. 30:251–278.

Wake, D. B. 2007. Climate change implicated in amphibian and lizard declines. Proc. Nat. Acad. Sci. 104:8201–8202.

Woods, C. 2005. Evaluation of VI-alpha and PIT-tagging of the seahorse *Hippocampus abdominalis*. Aquacult. Int. 13:175–186.

Herpetological Review, 2013, 44(2), xxx–xxx.

Using a Handheld PIT Scanner and Antenna System to Successfully Locate Terrestrially Overwintering Hatchling Turtles

The benefits of Passive Integrated Transponders (PIT) for identification of individual wildlife have been well documented. Originally used to determine movement of salmonids (Prentice and Park 1983), PIT technology has been used with a variety of reptiles and amphibians for spatial ecology studies. For example, mark-recapture techniques were used in conjunction with PITs to measure habitat use and movement of Brown Water Snakes (*Nerodia taxisplota*; Mills et al. 1995) and the activity patterns of arboreal geckos (*Gehyra variegata*; Gruber 2004). PITs do not transmit a signal and cannot be located unless an antenna is within close proximity.

While earliest studies with PITs in turtles involved sea turtles, PIT injection into the body cavity of smaller freshwater adult turtles was not considered until Buhlmann and Tuberville (1998) injected young *Trachemys scripta elegans* with 12 × 2 mm PITs in

NEIL DUNCAN
Department of Biology, Hofstra University, Hempstead, New York, and American Museum of Natural History, 79th Street at Central Park West, New York, New York 10024, USA
e-mail: duncan@amnh.org